

I claim:

1. A method for detecting biotinidase deficiency for newborn screening, comprising:

amplifying a DNA strand from a specimen to thereby form an amplification product; wherein

said amplification product is specific for detecting a mutation frequently observed in patients with

5 said biotinidase deficiency;

allowing a pair of labeled probes to hybridize to one strand of said amplification product,

wherein a detection probe is adapted to match to a sequence that may include said mutation, and an

anchor probe hybridizes to an adjacent sequence, thereby forming hybrids;

allowing fluorescence resonance energy transfer to occur between a donor fluorophore and

10 an acceptor fluorophore of each said hybrid, wherein an excitation wavelength of said donor

fluorophore and a fluorescence of said acceptor fluorophore is acquired; and,

generating a melting curve having peaks indicative of the melting temperature (Tm) of each  
said hybrid.

2. The method of claim 1, wherein said mutations are selected from the group consisting of

15 G98:d7i3, Q456H, R538C, D444H, and A171T.

3. The method of claim 1, wherein for the step of amplifying said DNA strand, such amplification is  
performed in an asymmetric manner.

4. The method of claim 1, wherein for the step of amplifying said DNA strand, a forward primer  
selected from the group consisting of those such sequences as set forth in SEQ ID NO: 4, SEQ ID

20 NO: 5, SEQ ID NO: 6, SEQ ID NO 7, and SEQ ID NO: 8 is used.

5. The method of claim 1, wherein for the step of amplifying said DNA strand, a reverse primer  
selected from the group consisting of those such sequences as set forth in SEQ ID NO: 9, SEQ ID  
NO: 10, SEQ ID NO: 11, SEQ ID NO 12, and SEQ ID NO: 13 is used.

6. The method of claim 1, wherein said detection probe is selected from the group consisting of those such sequences as set forth in SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO 21, SEQ ID NO: 22, and SEQ ID NO: 23.
7. The method of claim 6, wherein said detection probe is conjugated with LC red640.
- 5 8. The method of claim 7, wherein said detection probe is phosphorylated.
9. The method of claim 6, wherein said detection probe is conjugated with fitc.
10. The method of claim 1, wherein said anchor probe is selected from the group consisting of those such sequences as set forth in SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO 16, SEQ ID NO: 17, and SEQ ID NO: 18.
- 10 11. The method of claim 10, wherein said anchor probe is conjugated with LC red640.
12. The method of claim 11, wherein said anchor probe is phosphorylated.
13. The method of claim 10, wherein said anchor probe is conjugated with fitc.
14. The method of claim 1, wherein for the step of generating said melting curves, said fluorescence of said acceptor fluorophore is plotted against a temperature during a 35<sup>0</sup>-76<sup>0</sup> upward temperature ramp.
- 15 15. A method for detecting biotinidase deficiency for newborn screening, comprising:
  - amplifying a DNA strand from a specimen to thereby form an amplification product;
  - allowing a pair of labeled probes to hybridize to one strand of said amplification product, wherein one of said labeled probes is a detection probe selected from the group consisting of those such sequences as set forth in SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO 21, SEQ ID NO: 22, and SEQ ID NO: 23, and wherein one of said labeled probes is an anchor probe selected from the group consisting of those such sequences as set forth in SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO 16, SEQ ID NO: 17, and SEQ ID NO: 18; thereby forming hybrids;

allowing fluorescence resonance energy transfer to occur between a donor fluorophore and an acceptor fluorophore of each said hybrid, wherein an excitation wavelength of said donor fluorophore and a fluorescence of said acceptor fluorophore is acquired; and,  
5 generating a melting curve having peaks indicative of the melting temperature (Tm) of each said hybrid.

16. The method of claim 15, wherein for the step of amplifying said DNA strand, such amplification is performed in an asymmetric manner.

17. The method of claim 15, wherein for the step of amplifying said DNA strand, a forward primer selected from the group consisting of those such sequences as set forth in SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO 7, and SEQ ID NO: 8 is used.

18. The method of claim 15, wherein for the step of amplifying said DNA strand, a reverse primer selected from the group consisting of those such sequences as set forth in SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO 12, and SEQ ID NO: 13 is used.

19. The method of claim 15, wherein said detection probe is conjugated with LC red640.

15 20. The method of claim 19, wherein said detection probe is phosphorylated.

21. The method of claim 15, wherein said detection probe is conjugated with fitc.

22. The method of claim 15, wherein said anchor probe is conjugated with LC red640.

23. The method of claim 22, wherein said anchor probe is phosphorylated.

24. The method of claim 15, wherein said anchor probe is conjugated with fitc.